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14. ABSTRACT

The overarching hypothesis of this project was that GPR17 signaling results in blockade of remyelination in neuroinflammatory lesions. We thus predicted that GPR17 could serve as an important target for promoting remyelination in these lesions. The specific aims of this study were: (1) To delineate the role of GPR17 in murine models of demyelinating diseases; and (2) To test the therapeutic potential for GPR17 agonists and antagonists in two models of multiple sclerosis. Our studies demonstrate that GPR17-deficient mice developed less severe disease and recovered faster from paralysis. Moreover, these mice showed reduced CNS-targeted pathogenic immune responses. These results provide us a strong basis to pursue drug-based treatment for this disease in the future.

15. SUBJECT TERMS
EAE, GPR17, remyelination

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Table of Contents

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory, demyelinating disorder of the central nervous system (CNS) affecting over 400,000 individuals in the US. Although the etiology of the disease is unknown, it is believed to be an autoimmune disorder with an involvement of T-cell due to the characteristic pathology, T-cells in the CNS and modulation of clinical disease by immunotherapeutic agents (*1*). However, it is increasingly becoming clear that in addition to immunomodulation, therapeutic approaches aimed at promoting remyelination is essential for better management of the disease (*2*). Olig1, a bHLH transcription factor, promotes oligodendrocyte maturation and is required for myelin repair (*3, 4*). We have shown that one of the genes regulated by Olig1 is G-protein coupled receptor 17 (GPR17). GPR17 opposes the action of Olig1 thereby negatively regulating the differentiation of oligodendrocyte precursor cells (OPCs) in to mature oligodendrocytes (*5*). The signaling pathways involving GPR17 which culminates in the prevention of OPCs is not well understood. In our attempt to understand these pathways, we have observed that GPR17 expression is restricted to oligodendrocyte lineage cells and is down regulated during myelination and adulthood. Using transgenic mice, we also observed that sustained expression of GPR17 causes myelination defects whereas knockout of GPR17 leads to early onset of myelination during development. Interestingly, we observed that GPR17 is upregulated in the inflammatory demyelinating lesions in experimental autoimmune encephalomyelitis (EAE) a mouse model of MS. Further analysis revealed that GPR17 is upregulated in MS plaques as compared to the white matter from non-neurological donor samples and normal appearing white matter from MS donors. **These data suggest that GPR17 regulates the transition from immature to myelinating oligodendrocytes and hence may serve as a potential therapeutic target for CNS myelin repair. This study was mainly aimed at understanding biology of GPR17 in MS with an emphasis on developing novel combination therapeutic approaches for MS.**

BODY

This was a two-year study focused on the following two aims:

1. To delineate the role of GPR17 in murine models of demyelinating diseases.
2. To test the therapeutic potential for GPR17 agonists and antagonists in two models of MS.

As outlined in the statement of work (SOW), Dr. Karandikar's laboratory was responsible for experiments addressing the EAE model of MS. Below, we outline our research findings according to the tasks outlined in the SOW.

Task 1. Test the prediction that loss of GPR17 will diminish demyelinating pathology.

Study EAE in WT and GPR17-KO mice

To evaluate EAE in GPR17-KO mice we obtained breeding mice (4 generations backcrossed to C57BL/6) from Dr. Richard Lu's laboratory and initiated generation of GPR17-KO mice. The breeding strategy involved breeding GPR17$^{+/-}$ males with GPR17$^{+/-}$ females. Mice were routinely genotyped, using primers shown in Figure 1.

[A]

GPR17 KO Fwd	CAGCAGCCTCTGTTCCACATACA
GPR17 KO Rev	CACGAGTGAAGTCACTGAGTGTCT
GPR17 WT Fwd	CTGCTTCTACCTTCTGGACTTCATC
GPR17 WT Rev	AGGTGAGCATAGAGAGGTCTTCGA

[B]

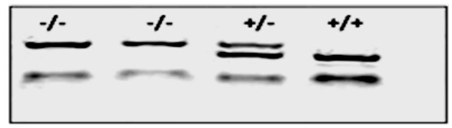


Figure 1. **Genotyping of GPR17 KO mice.** [A] Sequence of primers used to genotype GPR17 KO mice. [B] 10 µl from the 25 µl PCR reactions was run on a 1% agarose gel. +/+ sequence is identified by the presence of a 310 bp band where as a 380 bp band corresponds to -/-. Presence of both these bands represent +/- genotype. The low molecular weight bands are primer dimers.

Once mice were obtained, we initiated the studies of EAE in WT and GPR17-KO mice. For this, we induced active EAE by immunizing GPR17-KO mice and WT-C57BL/6J mice with myelin oligodendrocyte glycoprotein (MOG) derived peptide-MOG_{35-55}. We observed a significant difference in the disease severity between the two groups with GPR17-KO mice demonstrating lower disease grades (Figure 2). Interestingly, these mice also showed faster and enhanced recovery from the paralysis. These data suggest demonstrate an important role for GPR17 in EAE pathogenesis, supporting our underlying hypothesis.

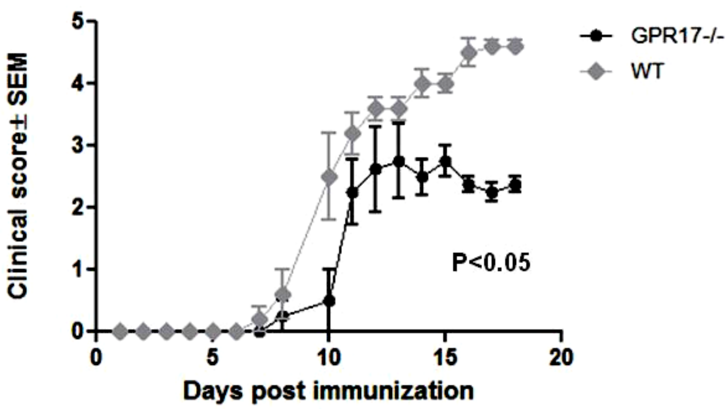


Figure 2. GPR17-/- mice develop significantly lower EAE severity. An emulsion of the peptide and Complete Freund’s adjuvant was made and the mice were subcutaneously immunized in the flanks with 100 µg of peptide on each site of immunization. Pertussis toxin (200 ng) was injected on the day of immunization and on day 2 of immunization. The mice were then monitored for daily for disease severity and were graded according to the following scale: 0, no paralysis; 1, loss of tail tonicity; 2, hind limb weakness; 3, partial hind limb paralysis; 4, complete hind limb paralysis; 5, hind limb paralysis and forelimb weakness/moribund state; 6, death. The grey lines correspond to disease scores of WT mice and the black lines to GRP17-KO mice. X-axis corresponds to the days following immunization and the Y-axis to the mean clinical score of the mice. Standard experimental mean was used to plot the error bars. Graphpad Prism 4.0 was used to plot the graph and calculate p value by applying Student’s t-test.

The reduced disease severity and greater recovery in GPR17-KO mice can be due to either increased remyelination or decrease in immune response against CNS-antigens. To address if the immune response is affected in GPR17-KO mice, we evaluated the T-cell response to MOG35-55 in vitro using a Carboxyfluorescein succinimidyl ester (CFSE)-based lymphocyte proliferation assay. Spleen cells from both the groups were isolated and stained with CFSE as previously described (*6*). Cells were then cultured in the presence of MOG_{35-55} for 5 days and the CFSE-dilution was monitored by flow cytometry on BD- LSR II flow cytometer.

We observed a decreased proliferation of CD4+ T-cells from GPR17-KO mice when compared to WT mice (Fig 3). This can be attributed to the reduced disease severity or can be a direct effect on the CD4+ T-cell functioning. Since CD4+ T-cells are the main auto aggressors in EAE, a suboptimal CD4+ T-cell response may prove to be beneficial in disease management. There was no significant difference observed in the proliferation of CD8+ T-cells. We are presently evaluating the effect of GPR17-deletion on immune responses by addressing T-cell functioning in terms of proliferation, cytokine production and disease induction.

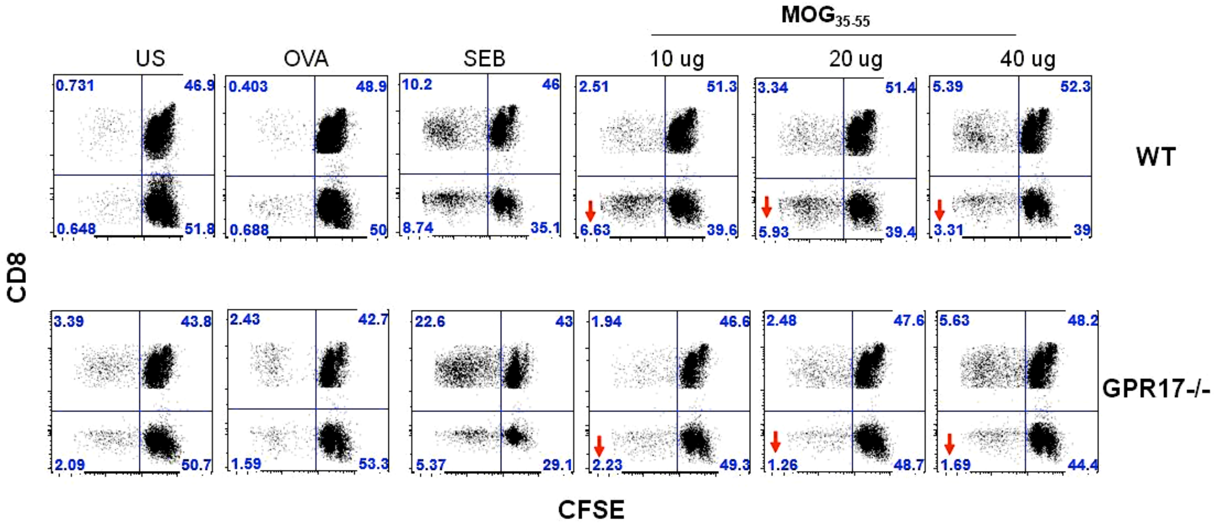
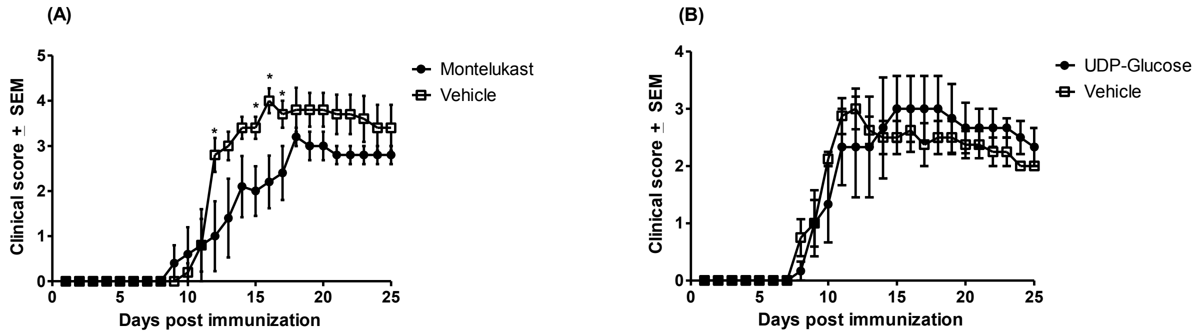


Figure 3. CFSE-based lymphoproliferation assay. Spleens from MOG_{35-55} immunized GPR17-/- and WT were harvested and cells were harvested and stained with CFSE. Subsequently, $1x10^6$ cells were added to FACS tubes and MOG_{35-55} was added at various concentrations. Staphylococcal enterotoxin B (SEB) was added at a concentration of 1 µg/ml as positive control for proliferation and $OVA_{323-339}$ peptide was used as peptide control. The cells were cultured in a CO_2 incubator for 5 days following which they were surface stained for CD4, CD8 and TCRvβ. CFSE-dilution was monitored by flow cytometry and the data was analyzed using FlowJo software. Live cells were gated on the basis of forward and side scatter profile following which T-cells were gated based on TCRvβ expression. X-axis corresponds to CFSE intensity while Y-axis represents CD8. The upper panel corresponds to proliferation of T-cells isolated from WT mice and lowers one to GPR17-/- cells. The red arrows indicate the percent proliferation of CD4+ T-cells in response to $MOG_{35-55.}$

Task 2. Test therapeutic potential for GPR17 antagonists.

Since GPR17-/- demonstrated significantly lower disease severity, we hypothesized that blocking the receptor would have a similar effect and hence can be a potential therapeutic approach for MS. To test this, we utilized the GPR17 antagonist, montelukast [already FDA-approved for other conditions], to study its effects on EAE. The disease severity of mice treated with montelukast was compared with the control group (treated with vehicle). As shown in Figure 4, mice receiving montelukast demonstrated a slight but significantlyy lower disease severity. However, when we attempted the converse by injecting mice with an agonist such as UDP-Glucose, we did not see an increase in the disease severity.

Optimization of montelukast dosing and use of alternative agonists are being attempted to address these concerns.



`**Figure 4. Effect of GPR17 antagonist and agonist on EAE severity.** EAE was induced by immunizing mice with 200 ug of $MOG_{35\text{-}55}$/CFA emulsion. 200 ng of Pertussis toxin was administered on day 0 and day 2. Disease severity was graded as described above. **(A)** 10 mg/kg of Montelukast in 10% ethanol was administered on day -1 and on alternate days for the course of the experiment. **(B)** 200 mg/kg of UDP-glucose was administered every other day starting day -1.

Task 3. Test role of GPR17 during GA therapy.

Task 3 turned out to be overambitious for a 2-year project. Based on results from Tasks 1 and 2, we are planning to develop a longer-term grant application to seek funding for testing and developing combination therapy for this disease.

KEY RESEARCH ACCOMPLISHMENTS

- Evaluated EAE in GPR17-KO mice in comparison to WT-mice and observed a lower disease severity in GPR17-KO mice. This establishes a role of GPR17 in EAE pathogenesis/recovery.
- Demonstrated that CD4+ T-cells from GPR17-KO respond less efficiently to $MOG_{35\text{-}55}$.
- Evaluated the effect of GPR17 agonist and antagonist on EAE severity and demonstrated that GPR17 antagonist treatment decreases EAE. There is need to refine the dosing and use alternative reagents to evaluate the best course of therapy.

REPORTABLE OUTCOMES

None thus far.

CONCLUSION

Our initial EAE studies carried out in GPR17-KO mice have demonstrated that GPR17 plays an important role in EAE pathology. In addition to a possible role in remyelination as seen by a lower disease severity (Fig 2), deletion of GPR17 also appeared to dampen the autoreactive CD4+ T-cell responses (Fig 3). Furthermore, mice treated with GPR17 antagonist demonstrated a lower disease severity. These data have encouraged us to pursue our study with the goal of developing a novel combination therapy. This goal was overambitious for a 2-year project, but we plan to develop grant applications to seek funding for these studies in the future.

BIBLIOGRAPHY AND PERSONNEL

No Publications/abstracts thus far.

Personnel Supported by this award:
Venkatesh Kashi, PhD, Postdoctoral Fellow
Wallace Baldwin, BS, research Assistant

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